

DNA MULTI-PROBE TEMPERATURE VERIFICATION OF THE MASTERCYCLERS

A. SCOPE

The Eppendorf Temperature Verification System (TVS) consists of a sensor plate with 15 temperature sensors, a system base for digitizing the temperature data, and a PC with a database and software for saving and evaluating the temperature data.

The Eppendorf Temperature Verification System performs the following tasks:

- Verifies the temperature control of the thermoblock and heated lid
- Provides certificates of conformity and measuring reports for the thermal cyclers
- Adjusts the temperature control of the thermoblock if the data generated during the verification process is outside Eppendorf's specifications

B. QUALITY CONTROL

- B.1 Protective gloves, scrubs, and a lab coat will be worn at all times when performing this procedure to prevent contamination.
- B.2 The Mastercyclers will have the temperature verification completed quarterly.
- B.3 The Eppendorf temperature verification system will be sent out for calibration every 18 months. After the Eppendorf temperature verification system is calibrated and returns to the laboratory, it will be visually inspected for damage. The calibration certification will be reviewed and stored in the LAM portion of BEAST.
- B.4 If there is a calendar year gap in calibration dates due to the 18 month span, then a performance check shall be carried out on the Eppendorf temperature verification system. (For example: System sent out for calibration in Oct. 2026 and again in April 2028, the performance check will be performed sometime in 2027).
- B.5 The Eppendorf temperature verification system can be used to make adjustments to the Mastercycler's block if the temperature deviations are greater than -2°C to +1°C of the set temperature. This will be followed by a performance check of the adjusted Mastercycler.
- B.6 The Eppendorf temperature verification system is safely stored and transported in the manufacturer's case provided with the instrument.

C. SAFETY

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- C.1 Protective gloves, and a lab coat will be worn at all times.
- C.2 The Mastercycler sample block may be hot immediately after the verification is complete. Handle the sensor plate with care.

D. EQUIPMENT

- D.1 Eppendorf Temperature Verification System (sensor, base, and PC)
- D.2 Mastercycler

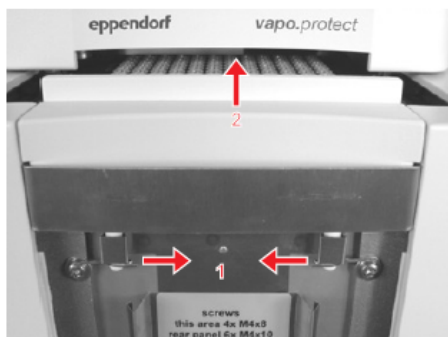
E. PROCEDURE

NOTE – BEFORE BEGINNING, MAKE SURE THAT THERE ARE NO PLATES ON ANY OF THE THERMAL CYCLERS THAT ARE TO BE VERIFIED.

- E.1 If cotter pins are present (may be present in new thermal cyclers or after shipping) follow steps E.2 thru E.4. If cotter pins are not present, continue to step E.5.
- E.2 Remove the front panel on all thermal cyclers using the front panel tool (See picture below, all pictures taken from the Eppendorf Temperature Verification Operation manual)



- E.3 Straighten the bent legs of the cotter pins and remove towards the middle of the device using pliers (1) (these will only be present in new instruments and will not be replaced unless the instrument is being shipped). Remove the front clip in an upwards motion (2) (See picture below).



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- E.4 Re-insert the front panel.
- E.5 On the back of the Mastercyclers, locate the two CAN address dials. These dials are circular with the numbers 0-9 printed on them and look like this:



Left Right

- E.6 (taken from Eppendorf operating manual)
- E.7 Using a small, flat-head screwdriver, rotate the right dial so that it is one digit greater than the number that the thermal cycler is labeled as. For example, for Cyclor 1 the CAN address should read 0 (left) 1 (right), before rotation and read 0 (left) 2 (right) after the rotation and during the verification. For Cyclor 2 the CAN address should read 0 (left) 2 (right), before rotation and read 0 (left) 3 (right) after the rotation and during the verification, etc.
- E.8 On the Eppendorf Mastercycler interface located on the front of Cyclor 1, scroll up to the top of the list so that the option "eppendorf" is highlighted.
- E.9 Press "Shutdown".
- E.10 A prompt will appear saying that shutting down the interface will cause a shutdown of all current runs. Press "Shutdown" again.
- E.11 Another prompt will appear on the interface to shutdown all thermal cyclers. Turn off all Mastercyclers at this time.
- E.12 Disconnect the interface and remove it from the front of Cyclor 1.

NOTE: BEFORE PROCEEDING, THE TEMPERATURE VERIFICATION SYSTEM AND THE CONNECTED LAPTOP MUST BE COMPLETELY POWERED DOWN.

- E.13 Connect the sensor plate to the SUB-D connection located on the back of the Temperature Verification System.

NOTE: WHEN NOT IN USE, THE SENSOR PLATE SHOULD BE STORED IN A STORAGE CLIP USED TO HOLD A 96-WELL PLATE.

- E.14 Insert the attached USB drive into the USB port located on the back of the Temperature Verification System. Make sure the golden-metal grooves of the flash drive are pointed upwards.
- E.15 Connect the Ethernet cable from the back of the Temperature Verification System into the CAN-IN port on the back of Cyclor 1.
- E.16 Plug in both the Temperature Verification System and the power supply for the laptop.
- E.17 Confirm the USB cable is connected to the laptop and the Temperature Verification System.

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- E.18 Turn on the laptop, followed by the Temperature Verification System, and finally the Mastercyclers.
- E.19 Open the Sensor Plate Info by going to My Computer and then selecting Sensor Plate (E). Allow blocked content.
- E.20 On the Desktop of the computer, double-click on the "Temperature Verification System" icon.
- E.21 To logon, use the username "administrator" and the password "Administrator".

NOTE: A PROMPT MAY APPEAR ASKING TO SPECIFY A COMPUTER INTERFACE. SELECT THE DEFAULT OPTION.

- E.22 After successfully logging in, the "Administrator" tab will open in the navigator window.
- E.23 Click on the "Cycler" tab.
- E.24 Click on the "Connect Eppendorf Cycler" button. A prompt will appear asking for the selection of a cycler to connect to. The following options should be available:

- TCYCLER 2
- TCYCLER 3
- TCYCLER 4
- TCYCLER 5

If not, double-check the CAN addresses are set correctly, power everything off, and reboot all instruments.

- E.25 Select "TCYCLER 2" and click "OK".
- E.26 Click on the "Verify" tab. To double-check that the correct Mastercycler has been selected, check to see if the serial number listed on this tab matches up with the serial number of the Mastercycler.
- E.27 Open the lid of the Mastercycler and remove the front clip.
- E.28 Place the sensor plate on the thermal block. Replace the front clip with the provided cable lead-through front clip so that the wire connected to the sensor plate will not be damaged. Close the lid to the Mastercycler. See below.

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NOTE: MAKE SURE THERE IS PLENTY OF SLACK ON THE WIRE THAT IS CONNECTED TO THE SENSOR PLATE.

- E.29 Click "Start Verification". A prompt will remind the user to make sure the lid is closed before starting. Click "OK".
- E.30 The verification will take six to ten minutes. After it has finished, click "OK". A results page will open automatically. If the verification fails, continue to the next step. If the verification passes, skip ahead to step E.35. If the verification fails, contact the DNA Technical Leader immediately. Do not adjust the block without the permission of the DNA Technical Leader.
- E.31 Click the "Report" button and click "Save As Text" in the Thermal Cycler Temperature Verification folder in the DNA folder on the K: drive. Name the file as such:

Ep#-fail-today's date OR Pro#-fail-today's date

Ep# denotes the Mastercycler Ep thermal cycler that failed the verification. Pro# denotes the Mastercycler Pro S thermal cycler report that passed the verification

- E.32 Click on the "Adjust" tab.
- E.33 Click "Start Adjustment". This should only take approximately one minute. After the instrument has been adjusted, a prompt will ask for the Mastercycler to be re-verified.
- E.34 Click the "Verify" tab and click the "Reset" button.
- E.35 Return to step E.23 and repeat the verification. If it fails more than once or any of the reports are +1 or -2°C of the expected temperature, the Mastercycler will be removed from service and Eppendorf technical support will be contacted.
- E.36 If the verification passes, click on the "Certificate" button, create a .pdf document, and save it in the appropriate Temperature Verification folder on the computer desktop. Name the file as such:

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Ep#-cert-today's date OR Pro#-cert-today's date

Ep# denotes the Mastercycler Ep thermal cycler that passed the verification. Pro# denotes the Mastercycler Pro S thermal cycler report that passed the verification.

Under User, type the initials of the analyst who performed the verification.

- E.37 Click the "Report" button and click "Save As Text" file in the appropriate Temperature Verification folder on the computer desktop. Name the file as such:

Ep#-today's date OR Pro#-today's date

Ep# denotes the Mastercycler Ep thermal cycler report that passed the verification. Pro# denotes the Mastercycler Pro S thermal cycler report that passed the verification.

- E.38 To save a certificate or report to a SD card or a USB flash drive, open the appropriate Temperature Verification folder on the computer desktop. Double click on the appropriate Mastercycler, i.e. EP1, EP2, etc. Right click on the saved certificate or report and select "Send To" and choose the appropriate SD card or USB flash drive.
- E.39 Click the "Cycler: Mastercycler ep (e.g. 96 positions)" tab.
- E.40 Click the "Disconnect Eppendorf Cycler" button.
- E.41 Repeat steps E.18-E.33 for the remaining three Mastercyclers, making sure to select the appropriate cycler when connecting.

NOTE: REPLACE THE ORIGINAL FRONT CLIP AFTER EACH VERIFICATION.

- E.42 After completing all verifications, remove the cable lead-through front clip from the last Mastercycler, remove the sensor plate from the thermal block, and return it to its storage clip. Replace the original front clip.
- E.43 Exit the Temperature Verification System program on the laptop and shutdown the computer.
- E.44 Power off the Temperature Verification System and the Mastercyclers.
- E.45 Disconnect the Ethernet cable attached to the back of the CAN-IN port on the back of Cycler 1.
- E.46 Using a small, flat-head screwdriver, return the Mastercyclers to their original CAN address using the dials located on the back of the Mastercyclers.
- E.47 Reconnect the Eppendorf Mastercycler interface to its original position and turn on all Mastercyclers.
- E.48 Transfer the certificates and reports for each Mastercycler from the SD card or USB flash drive to the Thermal Cycler Temperature Verification folder in the DNA folder on the K drive. Print each certificate and report and give these to the Technical Leader.

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F. INTERPRETATION GUIDELINES

Temperature (mean)	Mean value of the measured temperatures of the six inner block temperature sensors. This value is calculated separately for the target temperatures 35 °C, 72 °C and 95 °C. Limiting values are: <ul style="list-style-type: none">• 34.10 °C to 35.90 °C at 35 °C• 71.10 °C to 72.90 °C at 72 °C• 93.70 °C to 96.30 °C at 95 °C
Temperature homogeneity	Maximum deviation among the six inner block temperature sensors. This value is calculated separately for the target temperatures 35 °C, 72 °C and 95 °C. Limiting values are: <ul style="list-style-type: none">• 0.00 °C to 1.80 °C at 35 °C and 72 °C• 0.00 °C to 2.60 °C at 95 °C
Heating rate/cooling rate (mean)	Average heating and cooling rates, calculated using a linear regression of the temperature values measured during ramp phases between 40 °C and 90 °C.

The verification has passed when the limiting values for temperature accuracy and temperature homogeneity were observed on each of the six inner block temperature sensors. The measured values of the sensors on the edge of the block and in the corners, and the heating and cooling rate, are not incorporated in the verification – but are nevertheless included in the report. With **Info**, you receive additional information on the evaluation.

(Taken from the Eppendorf Temperature Verification System Operating Manual)

G. REFERENCES

G.1 Eppendorf Temperature Verification System Operating Manual, 2009.

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